Leica TCS SP2 UV

Brightfield, Phase Contrast and DIC

Focusing and centring the condenser:

(Note: Transmitted light adjustment = black wheel on bottom, left side of microscope; rotate towards you to increase light):

1) Place your slide on the stage (moving the condenser arm if necessary).
2) Using the MicCtrl button (in software) choose 'User'.
3) With the Obj button select the 10x objective (HC PL Fluotar 10x 0.3 DRY).
4) Select **BF** on condenser, and **BF** on silver wheel on left hand side of microscope just below the objectives.
5) Close the condenser iris diaphragm (to <2) and bring the sample into focus.
6) Close the field iris diaphragm (top) until you can see the edge of the diaphragm in the image plane.
7) Focus the condenser with the large silver, knurled knob (to left of condenser) until the edge of the diaphragm is sharp.
8) Centre the condenser using the two small silver, knurled knobs at the front of the condenser.
9) Open up the field iris diaphragm until the edge of the diaphragm **just** disappears from the field of view.

(Note: If you use a different objective in your experiments, you may want to make minor adjustments to the focus of the condenser by closing down the field iris diaphragm and then moving the condenser with the large silver, knurled knob.)

For Phase Contrast:

1) Open up the condenser aperture diaphragm to PH.
2) Choose appropriate Phase rings on condenser to match the objective, thus:
   a) HC PL Fluotar 10x 0.3 DRY = **PH1**
   b) PL Fluotar 63x 0.7 DRY = **PH2**
   c) PL Fluotar 25x 0.7 OIL = **PH2**
   d) HCX PL APO CS 40x 1.25 OIL = **PH3**

   (Note: The HCX PL APO U-V-I 40x 0.75 DRY and the HCX PL APO CS 63x 1.4 OIL are not a phase objectives.)
3) Select **BF** on silver wheel on left side of microscope just below objectives.
4) If you haven't already done so, bring sample into focus.

5) Using the MAG control button on front of microscope choose the Bertrand lens (B**)**, you should now see both sets of phase rings.
6) Focus the rings using the black wheel at the bottom, right hand side of the microscope (below the silver wheel labelled **B**).

(Note: If the rings are out of alignment consult PT or AW)

7) Using the MAG control return to 1x.

For DIC:

1) Open up the condenser aperture diaphragm to PH.
2) If you haven't already done so, bring sample into focus.

(Note: It is probably easier to do this in brightfield or phase before switching to DIC.)

3) Choose appropriate setting on condenser to match objective:
   a) HCX PL APO U-V-I 40x 0.75 DRY = **40**
   b) PL Fluotar 63x 0.7 DRY = **40/63**
   c) PL Fluotar 25x 0.7 OIL = **40**
   d) HCX PL APO CS 40x 1.25 OIL = **40/63**
   e) HCX PL APO CS 63x 1.40 OIL = **40/63**

   (Note: The HC PL Fluotar 10x 0.3 DRY is not a DIC objective)

4) Select lower polarizer (silver wheel on left side of microscope just below objectives) to match objective:
   a) HCX PL APO U-V-I 40x 0.75 DRY = **D**
   b) PL Fluotar 63x 0.7 DRY = **D**
   c) PL Fluotar 25x 0.7 OIL = **D**
   d) HCX PL APO CS 40x 1.25 OIL = **E**
   e) HCX PL APO CS 63x 1.40 OIL = **E**
5) Insert the upper polarizer in the transmitted light illumination path (above condenser - POL).
6) Insert half phase plate (left side of microscope, below objectives - ICT/P).
7) Adjust (rotate) upper and lower polarizers (lower = silver wheel on left hand side of microscope, below objectives labelled **D** or **E**) until image is optimal.

WARNING: If you want to do fluorescence work after using DIC, be sure to remove the half phase plate (ICT/P) and the lower polarizer (move silver wheel below objectives, on left hand side of microscope, to **BF**).